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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of:)
: Examiner: Michael V. Meller
MASANOBU HOKASE)
: Group Art Unit: 1651
Application No.: 09/581,758)
:
Filed: June 16, 2000)
:
For: METHODS FOR PREVENTING)
AND TREATING MASTITIS :

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION PURSUANT TO 37 C.F.R.1.132

I, Ryoji ONODERA, of 558-18 Kifuji, Shimokitakata-cho, Miyazaki-shi, Miyazaki, Japan, do hereby declare as follows:

I received my first bachelor degree in the Department of Agriculture, at University of Tokyo in 1967. Since 1999 I have been the Doctor of Agriculture, Professor of Agriculture, Faculty of Agriculture of Miyazaki University. I received many honorable awards, e.g., the Prize of the Association of Japanese Agricultural Scientific Societies in 2001 et al.

I have a full knowledge of the present invention and cited references.

I order to show the differences between the subject matter of the references and the subject matter of the application, the following experiments were undertaken by me. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Correlation between increases in somatic cell count in milk and mastitis

Mastitis in mammals is inflammation, which is caused by invasion and proliferation of bacteria, etc. into the udder, and occurs in the mammary duct system and mammary gland tissues. When mammals have the mastitis, inflammation inhibits the synthesis of milk (cow's milk), so that the mammals secrete abnormal milk containing leukocytes, etc. Further, damaged mammary gland cells become contracted or cause the growth of connective tissues, so that tissue cells are abraded and then excreted into milk.

Leukocytes and tissue cells, etc. contained in milk are generally referred to as "body cells." Body cell count (BCC) in milk is known as a simple universal indicator of the onset of mastitis.

An increase in body cell count is closely linked to microbial contamination of milk. Thus, dairy companies include the body cell count in their quality standards for buying bulk milk*.

Body cell count in bulk milk produced from healthy cows is generally thought to be less than 200,000 cells/ml. Body cell count of 200,000 cells/ml or more indicates an extremely high possibility that cows having "potential mastitis," or which are likely to develop mastitis, are present in the group of cows. Bulk milk with a body cell count of more than 500,000 cells/ml suggests that there is a high probability that cows having mastitis are present in the group, so that in most cases, the bulk milk is discarded.

*Bulk milk: Milk produced by each cow is collected by the farm or cowhouse, and then stored in a tank. The collected raw milk is called "bulk milk" or "composite milk."

References

Duane N. Rice and Gerald R. Bodman (1993): The somatic Cell Count and Milk Quality
http://www.ianr.unl.edu/pubs/dairy/g1151.htm
Winston Ingalls (2001): Somatic Cells, Mastitis and Milk Quality
http://www.moomilk.com/archive/u-health-20.htm
Robert J. Harmon (2001): SOMATIC CELL COUNTS: A PRIMER
http://www.nmconline.org/articles/sccprimer.pdf

Validity of Field Test Data Shown in Examples 1, 2, 3 and 7 of Specification

Considerable labor and cost are required to collect milk from each cow, which is a large animal. Thus, in the field of animal industry, evaluating cows as "a group of cows" can be said to be a normal evaluation method.

In each of the Examples shown below, it is assessed that body cell count in bulk milk was 250,000 cells/ml, meaning that a group of cows diagnosed as having "potential mastitis" was selected.

In Example 1, the body cell count in the milk of a group of cows, initially having that of 250,000 cells/ml and thus indicating potential mastitis, decreased to 100,000 cells/ml or less when phytase was administered. This result suggests that the body cell count in the milk of cows having "potential mastitis" and being likely to develop mastitis decreased by administration of phytase. In other words, the onset of mastitis could be prevented by administration of phytase.

In Example 2, the body cell count in the milk of the test group administered with phytase clearly decreased. In contrast, the body cell count in the milk of the control group (not administered with phytase) increased to a level significantly suspected of indicating the possibility of potential mastitis. Besides, 3 cows developed mastitis and were removed from the production line. That is, these results absolutely suggest that the actual onset of mastitis could be prevented, and also suggest recovery whereby cows with "potential mastitis" became normal cows. Hence, the results show that mastitis could be prevented by administering phytase.

Example 3 is a comparison test of phytase and zinc methionine sulfate (Feedstuffs, 57, 52 p11, 1985) that has been thought to have a preventive effect on mastitis (involving a suppressive effect on body cell count). The body cell count of the control group administered with zinc methionine sulfate had decreased to 120,000 cells/ml, the normal body cell count, 2 months after the start of administration, while that of the test group administered with phytase had decreased to 150,000 cells/ml 1 month after the start of administration. The body cell count of the control group was 200,000 cells/ml or more at 1 month after the start of administration, which led to a diagnosis of potential mastitis. From these results, it can be said that phytase is clearly superior to zinc methionine sulfate in its preventive effect on mastitis.

In Example 7, the body cell count in the bulk milk was clearly decreased by

administration of composition C containing phytase. Although there were time periods during which composition C was not administered, fo r both groups A and B, the body cell count decreased to 180,000 cells/ml, which can be said to be a normal value, at week 6 (group A) and at week 8 (group B), respectively. These results suggest that the body cell count was suppressed by composition C containing phytase that is, recovery took place whereby cows with "potential mastitis" became normal cows. Thus the preventive effect of phytase-containing feed is evident. Further, reproducibility of the effect was confirmed in Example 7 by conducting the tests with the switchback procedure.

Though the tests were conducted at different farms, all the results of the above examples suggest the preventive effect of phytase on mastitis. Therefore, the credibility of these test results suggesting that mastitis in cows can be prevented by administration of phytase is high.

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